

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C08J 7/12, A61L 27/00 // C08L 67/04	A1	(11) International Publication Number: WO 97/05193 (43) International Publication Date: 13 February 1997 (13.02.97)
(21) International Application Number: PCT/IT96/00149 (22) International Filing Date: 24 July 1996 (24.07.96) (30) Priority Data: VR95A000068 28 July 1995 (28.07.95) IT (71) Applicant (for all designated States except US): SANITARIA SCALIGERA SPA [IT/IT]; Via della Consortia, 2, I-37137 Avesa (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): ZUCCATO, Alessandro [IT/IT]; Rigaste S. Zeno, 23/c, I-37123 Verona (IT). PEREGO, Gabriele [IT/IT]; Via Fossigalli, 3, I-10015 Ivrea (IT). CELLA, Gian, Domenico [IT/IT]; Via Minghetti, 1, I-28100 Novara (IT). (74) Agent: SANDRI, Sandro; Europatent s.a.s., Via Locatelli, 20, I-37122 Verona (IT).		(81) Designated States: AU, BG, BR, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KR, LT, LV, MX, NO, NZ, PL, RO, SG, SI, SK, TR, UA, US, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: A PROCESS OF SURFACE ACTIVATION OF BIOCOMPATIBLE AND BIOABSORBABLE ALIPHATIC POLYESTERS AND POLYESTERS THUS ACTIVATED (57) Abstract <p>A process of surface activation of a material based on one or more of the copolymers of lactic acid and ϵ-caprolactone is carried out on bioabsorbable and biocompatible aliphatic polyesters chosen from the group formed by homo- and copolymers derived from L-lactide, D-lactide, racemic D,L-lactide, meso D,L-lactide, ϵ-caprolactone, glycolide, δ-valerolactone, p-dioxanone, preferably copolymers of L-lactide, D-lactide, racemic D,L-lactide, meso D,L-lactide with ϵ-caprolactone; the process consists of performing at least one of the following treatments: acid or base hydrolysis, treatment by means of cold plasma, by chemical reactions or electromagnetic radiations. The process brings about a substantial increase in surface hydrophilicity and, consequently, a significant lowering of the wetting angle measured with water at 23 degrees Celsius in air. This can in many cases favour cellular adherence to bioabsorbable medical prostheses realised using these materials, and thus surface-activated, such as for example endothelialisation processes in vascular grafts.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

A PROCESS OF SURFACE ACTIVATION OF BIOCOMPATIBLE AND BIOABSORBABLE ALIPHATIC POLYESTERS AND POLYESTERS THUS ACTIVATED.

5 The invention relates to surface-activated copolymers of lactic acid and ϵ -caprolactone and to a procedure for preparation thereof.

 In recent times homo- and copolymers of lactic acid have been the subject of growing attention regarding their potential applications, in part already put into practice, in the biomedical sector.

10 Such applications, which take advantage of the biocompatibility and biodegradability of these materials, comprise among other things the manufacture of prosthetic implants, suture threads, microspheres for vehicling of principal active ingredients.

 A relative advantage of these materials is that of eliminating the need for a second surgical intervention, sometimes necessary for removing the implant, thanks to the gradual
15 biodegradability which favours regeneration of the pre-existing tissues.

 This advantage has been exploited, for example, in the preparation of bioabsorbable suture threads using homo- and copolymers of glycolic acid, commercially known as DEXON (mark of the company Davis and Jeck) and VICRYL (mark of the company Ethicon). In more recent times commercialisation has begun of the first products for the
20 orthopaedic sector, known as BIOFIX (mark of Bioscience Oy) and ORTHOSORB (mark of the company Johnson and Johnson).

 A field of use of bioabsorbable materials which has great use potential is the preparation of vascular grafts. Implants at present available, often made in DACRON (a material provided by the company Du Pont of Nemurs), as they are not bioabsorbable, lack
25 in some important requisites, such as for example a complete absence of thrombogenicity of the internal wall, or elastic properties which remain unaltered over time [New Polymeric Mater, 1 (2), 111-126 (1988)]. These limitations become important especially when prostheses are below 6mm diameter, which often leads during surgery to implant of autologous veins to repair small-caliber arteries, with all the problems deriving there-from.

30 The development of a biodegradable vascular prosthesis, which would thus promote the regeneration of pre-existing tissue, is of great interest and is at present the object of many studies for example, see Colloid Polym. Sci 264, 854-858 (1986) , but is also hindered by the problem of thrombogenicity for small calibers.

Thrombotic formation on the internal wall of the prosthesis happens especially during the initial implant phase, that is before the wall has been covered with endothelial cells, for which reason a biocompatible and bioabsorbable material for the manufacturing of a vascular graft would have a relevant advantage if it could have surface alterations aimed at accelerating the endothelialisation process.

The copolymers of lactic acid (L-lactic) and ϵ -caprolactone, developed in 1975 by Gulf Oil see US Patent US-A-4,057,537 (Sinclair) are very interesting materials for various biomedical uses, among which the realisation of nerve guides Biomaterial 15 (3), 189-193 (1994), J.Mater Sci. Mater in Medicine 4, 521-525 (1993), suture threads and bioabsorbable vascular prostheses.

The interest in these materials is linked, other than for their biocompatibility and bioabsorbability, to their excellent elastic properties (Polymer Bulletin 25, 335-341 (1991)) which are stable over time, especially for copolymers with about equal components of two comonomers (Makromol Chem 194, 2463-2469 (1993)).

These materials, on the other hand, have the drawback as far as biomedical use is concerned of having a low hydrophilicity, which reduces their wettability and the adherence of the organism cells, namely the endothelial cells. For the poly (L-Lactide-co- ϵ -caprolactone) at 50/50 weight, for example, the wetting angle measured with distilled water in air at 23°C is 82°, thus very high.

An aim of the present invention is to provide a process aimed at surface modification of a material based on one or more of the copolymers of lactic acid and ϵ -caprolactone such as to bring about a substantial increase in surface hydrophilicity and, consequently, a significant lowering of the wetting angle measured with water at 23 degrees Celsius in air.

This can in many cases favour cellular adherence to bioabsorbable medical prostheses realised using these materials, and thus surface-activated, such as for example endothelialisation processes in vascular grafts.

A further aim of the present invention is that said treatment is also aimed at reducing autoadhesiveness of said material in order to achieve considerable advantages in the manipulation and use of films, medical prostheses and the like.

According to a first aspect of the present invention a process of surface activation is provided of bioabsorbable and biocompatible aliphatic polyesters chosen from the group formed by homo- and copolymers derived from L-lactide, D-lactide, racemic D,L-lactide, meso D,L Lactide, ϵ -caprolactone, glycolide, δ -valeroactone, p-dioxanone, preferably

copolymers of L-lactide, D-lactide, racemic D,L lactide, meso D,L-lactide with ϵ -caprolactone, which consists of performing at least one of the following treatments: acid or base hydrolysis, treatment by means of cold plasma, by chemical reactions or electromagnetic radiations.

5 Advantageously, the process of hydrolysis is conducted in the presence of an aqueous solution of a base or an acid to accelerate surface reaction, inasmuch as excessively long processes of activation can induce a reduction in molecular weight and thus in the mechanical properties of the material.

10 At present preferred is the use of an aqueous solution of a base which constitutes a preferential condition inasmuch as it has demonstrated greater activity.

Suitable bases for obtaining watery solutions suited to the aim are, for example, strong alkalis, such as LiOH, Ba(OH)₂, Mg(OH)₂, NaOH, KOH, Na₂CO₃, Ca(OH)₂ and the weak bases, such as for example NH₄OH and the amines such as methylamine, ethylamine, diethylamine and dimethylamine.

15 Acids suitable for surface hydrolysis treatments can be chosen, for example, from among HCl, HClO₃, HClO₄, H₂SO₃, H₂SO₄, H₃PO₃, H₃PO₄, HI, HIO₃, HBr, lactic acid, glycolic acid.

Surface activation by means of hydrolysis can be conducted at temperatures preferably comprised between 0 degrees Celsius and the material softening temperature.

20 In the case of the 50/50 poly(L-lactide-co- ϵ -caprolactone), suitable temperatures are, for example, comprised between 10° and 60°C, preferably between 30° and 50°C.

Surface hydrolysis treatment is followed by careful washing to remove all traces of acid or base.

This surface hydrolytic treatment has been found to:

- 25 - produce a relevant surface change accompanied by a lowering of the wetting angle measured with water at 23°C in air, which in the case of poly(L-lactide-co- ϵ -caprolactone) at 50/50 weight passes from 82° to 30°C;
- 30 - modify morphologically the surface of the material with the creation of numerous microporosities, useful in cell seeding processes, such as for example endothelialisation;
- substantially reduce autoadhesivity of the material, whose shear detachment force changes from an initial 10Kg/cm to 0 Kg/cm following colonisation, with considerable advantages in manipulation and use of films, medical prostheses, etc.;
- unexpectedly, the viscosity inherent in the copolymer during the process of activation does

not undergo appreciable variation, such as to reduce its mechanical properties.

The process according to the invention is carried out using homo- or copolymers of lactic acid, preferably with the copolymers of lactic acid and ϵ -caprolactone.

5 These copolymers can be obtained, as is known, by means of polymerisation by ring opening polymerisation of the cyclic dimer ring of lactic acid (L-lactic, in the case of L-lactic acid) and ϵ -caprolactone, at temperatures generally comprised between 100°C and 200°C and in the presence of a suitable catalyst, such as for example Sn octanoate $\text{SnC}_8\text{H}_{15}\text{O}_2$, $\text{Al}(\text{iOPr})_3$, etc.

10 The copolymers of lactic acid and ϵ -caprolactone are suitable for working with the common methodologies used in heat-forming of plastic materials, such as for example, extruders, injection-moulders and so on.

15 These copolymers are also soluble in various organic solvents, among which, for example, methyl acetate, ethyl acetate, methylene chloride, chloroform, etc., for which reason many manufactured products, for example canicular prostheses, can be obtained by exploiting this solubility, with known processes, such as for example deposition, spreading, etc,

Plasma treatment can be carried out both in the presence of a reactive gas, for example air, Ar, O_2 with the formation of surface activation of oxygenate type, such as -OH, -CHO, -COOH.

20 Surface treatment, whether hydrolytic or with plasma, can remain unaltered or can be followed by further chemical modifications. Thus, for example, the COONa groups generated by a base hydrolysis can be subsequently converted into COOH groups by treatment with strong mineral acids.

25 Further, the surface freeing of alcoholic groups by means of a hydrolysis process can be followed by reaction by means of the addition of a compound provided with functional group or groups able to react with surface alcoholic groups, such as for example by means of the addition of an anhydride such as succinic anhydride, with the conversion of -OH groups into -O-CO-CH₂-CH₂-COOH groups.

30 In a further aspect of the present invention, prosthetic implants are provided which are realised with homo and copolymers of lactic acid, surface-activated for obtaining biocompatible and biodegradable materials. An example thereof is constituted by a partially bioabsorbable vascular prosthesis.

The process according to the invention is described in more detail in the following.

with reference to specific examples of practical realisation.

Example 1

Preparation of poly (L-lactide-co-ε-caprolactone)

5 L-lactide was synthesised and recrystallised 3 times by methyl anhydrous isobutyl ketone, dried under vacuum at 45°C for 24 hours and kept in a nitrogen atmosphere. ε-caprolactone, provided by Fluka, was dried on CaH₂, distilled at low pressure just before use and was kept under nitrogen. Hexane, provided by Farmitalia Carlo Erba, was distilled on LiAlH₄ and kept under nitrogen.

10 200 g of L-lactide, 200 g (186ml) of ε-caprolactone and an stirring magnet were placed in a preferably silanised (with Si(CH₃)₂Cl₂) 500ml two-neck flask. The flask was immersed in an oil bath whose temperature was gradually brought to 130°C, homogenizing the system as soon as made possible by the fusion of the lactide, by means of electromagnetic stirring.

15 A solution of 1.19g of Sn octanoate in hexane was prepared separately, up to a volume of 10ml, from which a measure of 2.13 ml was removed, which was equal to a content of 0.254g of Sn octoanate (having a catalysing function), which was then added to the monomer mixture. About 45 minutes after addition of the catalyst, whose quantity corresponded to a molar monomer/catalyst rapport of 5000, the electromagnetic stirring of the mixture became ineffective due to the system viscosity.

20 The reaction was protracted for 24 hours, thus obtaining an elastic transparent material having an inherent viscosity measured in chloroform at 25°C of 3.80dl/g and a residual monomer content of 2.3%, as estimated by TGA (thermogravimetric-isotherm analysis at 150°C for 60 minutes).

Example 2

Preparation of discs in 50/50 poly(L-lactide-co-ε-caprolactone)

25 A 10x10cm steel template was used, located between two Mylar sheets, in which 8 g of poly (L-lactide-co-ε-caprolactone) at 50/50 weight were placed.

30 Pressing was performed with three preheating minutes and 2 compression minutes at 50Kg/cm at a temperature of 160°C. Thus copolymer sheets were produced, having a thickness of 500 micron, from which 22mm diameter discs were die-cut. These discs exhibited a wetting angle with water, measured at 23°C in air, of 82°. The inherent viscosity of the material, determined in chloroform at 25°C, at a concentration of 0.2 g/dl, by Ubbelohde friction-tube viscometer, was 3.04 dl/g.

introduced, as described in example 3. The flask was kept at a temperature of 20°C, slowly stirring the contents by means of an electromagnet for 1 hour, then the acid solution was removed and numerous washes with distilled water were carried out over a period of 4 hours.

5 The discs of material thus activated exhibited a wetting angle with water of 47°, measured at 23°C in air.

Figure 1c is a microphotograph obtained by scanning electron microscope (SEM) at a magnification of 1500x, which illustrates the surface morphology of a disc in poly(L-lactide-co-ε-caprolactone) activated by base hydrolysis with NaOH and acid hydrolysis by
10 HCl.

Example 6

Conversion of -COONa surface groups into -COOH groups

80 ml of 3% HCl aqueous solution were placed in a 100ml flask and a 50/50 poly (L-lactide-co-ε-caprolactone) disc was introduced, as described in example 4. The flask was
15 immersed in a thermostatised water bath at a temperature of 20°C, slowly agitating the contents by means of an electromagnet for 1 hour, then the acid solution was removed and numerous washes with distilled water were carried out over a period of 4 hours.

The discs of material thus activated exhibited a wetting angle with water of 70°, measured at 23°C in air.

20

Example 7

Activation by means of cold plasma.

A parallel flat plate Gambetti plasma treatment reactor with capacitive coupling was used. The radiofrequency the machine operated at was 13.6 Mhz.

Activation was carried out on discs of 50/50 poly(L-lactide-co-ε-caprolactone)
25 having a thickness of 500 micron and a diameter of 22mm, prepared as described in Example 1.

These discs were applied with plasmas of air, oxygen and argon at a temperature of 20°C, at 20 and 50 Watts, for times of 30 and 60 seconds. The copolymer discs thus activated exhibited wetting angles with distilled water in air comprised between 31° and 55°,
30 as reported in Table 1 hereinbelow.

It can be observed that the variation in treatment times, from 30 seconds to one minute, had a relevant effect only in the case of the low-potential plasmas used (20W), while with 50W the surface activation with the plasmas used seems to have reached a limit

level after 30 seconds of treatment.

In the case of air, 50W potential plasma activated the surface of the material to a greater degree (32° - 31°), with respect to 20W plasma (54.5, 47). In the case of oxygen and argon plasmas no significant differences were observed between the different potentials.

5 Among the experimental conditions adopted, therefore, a 50W potential air plasma produced the greatest activation. This can be explained by the fact that the inert part of air, especially nitrogen, generates numerous radicals on the surface of the material, which is followed by a reaction with oxygen molecules and the formation of activated groups of oxygenate type such as -OH, -CHO, -COOH.

10

TABLE 1

Surface activation of 50/50 poly (L-lactide-co-ε-caprolactone) discs with cold plasma.

SAMPLE	GAS PLASMA	POTENTIAL (W)	TIME (SEC.)	WETTING ANGLE.
1	air	20	30	54,5
2	air	20	60	47
3	air	50	30	32
4	air	50	60	31
5	oxygen	20	30	52
6	oxygen	20	60	44,5
7	oxygen	50	30	47
8	oxygen	50	60	55
9	argon	20	30	55
10	argon	20	60	46
11	argon	50	30	47
12	argon	50	60	47

Example 8

15

Measures of Material Autoadherence

We proceeded as in examples from 3 to 7 and the copolymer was successfully compression molded using a 5x5cm steel template having a thickness of 1mm, located between two Mylar® sheets, wherein were placed 3g of 50/50 poly(L-lactide-co-ε-caprolactone).

The compression molding was executed with 3 minutes of preheating and 2 minutes of compression at 50Kg/cm at a temperature of 160°C. Sheets of copolymer were thus prepared, having a thickness of 1mm, from which strips of 50x10x1mm were cut.

5 The test pieces for the autoadherence measurements were produced by superposing by 10mm the ends of two copolymer strips. The measurement of the shear force was effected immediately after pressing by using an Instron model 4501 dynamometer at a temperature of 23°C and in relative humidity of 50%. This calculation, effected at a velocity of 200mm/min., provided the force needed to cause shear detachment of the two surfaces, equal to 10Kg/cm for the non-modified copolymer.

10 The strips of surface-activated poly(L-lactide-co-ε-caprolactone) did not give rise to any adherence in the above-reported conditions.

Figure 2 is a SEM 100x microphotograph illustrating the surface morphology of a non-activated poly(L-lactide-co-ε-caprolactone) disc and seeded with endothelial cells from a microcirculation of human fatty tissue: 4 days of culture.

15 Note the presence of few cells having a prevalently rounded shape (some of which are indicated by arrows).

Example 9

Cell Culture on non-activated discs.

20 Discs of 50/50 poly(L-lactide-co-ε-caprolactone) having a thickness of 500 micron and a diameter of 22mm were subjected to sterilisation with ethylene oxide and kept in sterile containers up to the moment of cell seeding.

For the seeding human endothelial cells were used, isolated from the microcirculation of human fatty tissue, removed from the abdominal wall of patients subjected to surgical operation for abdominal aneurysm.

25 Variable quantities (10-20g) of fatty tissue were snipped using small scissors and transferred into an Erlenmeyer flask containing Collagenase Type I 0.2% (SIGMA - St. Louis Missouri USA) in PBS (Phosphate Buffer Saline pH 7.4).

30 After an incubation period of 30 minutes at 37°C, constantly shaken, the floating adipocytes were eliminated and the residual cellular suspension centrifuged for 10 minutes at 200 x g.

The resulting pellet was re-suspended in PBS (Phosphate Buffer Saline pH 7.4) containing 0.1% of BSA (Bovine Serum Albumin fraction V IgG free, Low endotoxin) (SIGMA) and the cellular suspension obtained was subjected to filtration through a nylon

filter having a porosity of about 200 micron. After a further wash and centrifugation the pellet was re-suspended in 2ml of PBS/BSA and delicately layered on 8ml of Percoll 45% (mark of the company SIGMA) in PBS (280mOsm) and centrifuged at 1500 x g for 20 minutes.

5 The microvascular endothelial cells were recovered in a milky layer on the surface of the Percoll (SIGMA). After additional washes, the microvascular cells were re-suspended in Medium 199 25 MM Hepes (GIBCO BRL, Paisley, Scotland) containing 20% FCS (Foetal Calf Serum) (GIBCO BRL), 50g/ml ECGF (Endothelial Cell Growth Factor) (SIGMA), 100 U/ml heparin (SIGMA), 100 U/ml of penicillin (GIBCO BRL) and 100g/ml of streptomycin
10 (GIBCO BRL) and subjected to hemocytometer count and Trypan Blue Stain exclusion test (Flow Laboratories, Irvine, Scotland) for evaluation of cell vitality.

 All the operations described were carried out in conditions of total sterility under a laminar flow hood, and using sterile single-use glass and plastic materials. The poly(L-lactide-co-ε-caprolactone) discs were transferred, after repeated washes in sterile PBS
15 (Phosphate Buffer Saline pH 7.4) in adequate size Cell Wells 12 well plate, well diameter 22 mm - CORNING, New York USA). On each disc, lying at the bottom of the wells, from 1 to 2 x 10 cells per cm were seeded in about 3ml of complete Medium 199 (SIGMA).

 The culture was left for 4 to 6 days in an incubator at 37°C in a 5% CO₂ atmosphere.

 The discs were kept 4 days under culture and then fixed in 2.5% glutaraldehyde
20 (BAKER, Deventer, Holland) in cacodylate buffer (CARLO ERBA, Milan) and 1% osmic acid anhydrite (BAKER) in the same buffer, dehydrated in a growing series of ethanol (CARLO ERBA) and air-dried. Then they were subjected to analysis by means of scanning electron microscope.

 On non-activated lactic acid copolymer discs only rare cells were visible, not
25 numerically evaluable, and with a prevalence of rounded shapes (indicating poor adherence to substrate).

Example 10

Cell culture on NaOH activated discs.

 Isolation of the endothelial cells and the cell culture were performed using the
30 modalities described in example 9 above.

 The SEM analysis of the lactic acid copolymer discs subjected to base hydrolysis enable observation of the adherence of a certain number of endothelial cells, not exactly quantifiable, which, at the same, exhibited aspects of better adherence to the substrate (flat

cells).

Figure 3 is an SEM X 100 microphotograph illustrating the surface morphology of a poly(L-lactide-co-ε-caprolactone) disc activated with NaOH and seeded with endothelial cells from the microcirculation of human fatty tissue: 4 culture days.

5 The microphotograph shows the presence of flat cells (indicated by arrows in figure 3).

Example 11

Cell culture on discs activated with NaOH/HCl.

10 Isolation of the endothelial cell and the cell culture was achieved using the modalities described in example 9. The SEM analysis of the discs subjected to base hydrolysis and further activated with HCl, allowed identification of a relevant number of endothelial cells (approximately $8 \times 10^3/\text{cm}^2$) with flat-spindled morphology, which is an indication of good adherence to the substrate.

15 Figure 4 is a SEM X 100 microphotograph showing the surface morphology of a disc in poly(L-lactide-co-ε-caprolactone) activated with NaOH and HCl and seeded with endothelial cells from the microcirculation of human fatty tissue: 4 culture days. The presence of numerous flat cells can be observed (indicated with arrows in the figure).

The results of the surface activation of 50/50 p(LLA-CL) discs are reported in Table 2.

20

TABLE 2

Sample	reactive base	reactive acid	temperat. of treatment(°C)	wetting angle (°)	viscosity iner. (dl/g)
I	-	-	-	82	3,04
II	NaOH 5%	-	40	30	3,00
III	NaOH 5%	HCl 3%	20	47	-
IV	NaOH 5%	-	20	48	3,02
V	NaOH 5%	HCl 3%	20	70	-

Example 12

Cell culture on plasma: air 50W 1' surface activated discs.

25 Isolation of the endothelial cells and the cell culture were achieved using the modalities for example 6.

The SEM analysis of the lactic acid copolymer discs subjected to activation by oxygen at 50W for 1' evidenced a semiconfluent single layer of cells with prevalently spindled morphology.

5 The cells appear particularly flat, a characteristic which permits sight, in transparency, of both the cytoplasmic organoids and the nucleus and sometimes even the nucleolus - see photograph of figure 5.

Example 13

Cell culture on plasma: 02, 50W 30'' surface activated discs.

10 Isolation of the endothelial cells and the cell culture were achieved using the modalities for example 6.

The SEM analysis of the lactic acid copolymer discs subjected to activation by oxygen at 50W for 30'' evidenced a semiconfluent single layer of cells with prevalently elongate and flattened morphology. In some zones the cells appear less frequent and relatively large areas of polymer are left uncovered- see photographic reproduction of figure 15 6.

Example 14

Cell culture on plasma: Ar, 50W, 1' surface activated discs.

Isolation of the endothelial cells and the cell culture were achieved using the modalities for example 6.

20 The SEM analysis of the lactic acid copolymer discs subjected to activation by argon at 50W for 1' evidenced a semiconfluent monolayer of spindled and particularly flat cells - see photograph of figure 7.

This characteristic possibly represents the morphological equivalent of a more tenacious cell adherence to the non-activated substrate.

Example 15

Preparation of a partially bioabsorbable vascular prosthesis

the prosthesis was prepared with poly(L-lactide-co-ε-caprolactone) 50:50 by weight, synthesized as described in the example 1. A sample of 8.0 g of this copolymer was dissolved in anhydrous methyl acetate, to a volume of 100 ml. A rotating glass bar, fixed at one end in a mechanical stirrer and having a diameter of 6.0mm, was immersed in the 30 solution. The bar was then slowly pulled out, so obtaining a uniform coating and the solvent was then evaporated at room temperature, with a gentle air stream. After having turned upside the glass bar, the operation was repeated to get a second layer and so on to prepare a

prosthesis having the desired thickness, of 300 micron. The prosthesis, having a length of 10 cm, was dried under vacuum at room temperature and kept under nitrogen.

Alternatively the prosthesis was obtained by operating with an extruder having a screw with a length of 30 cm. The extrusion was performed at a speed of 40 rpm and at
5 temperatures of 130°C, 150°C, 165°C, 170°C, 140°C, respectively from the first zone to the head of the extruder. The desired wall thickness was obtained by varying the flux of air insufflated in the extruder head and the take up speed.

The prosthesis prepared with one of the above described methods was at last wrapped with a Dacron® reinforcement, in the form of a stitch, to improve the mechanical
10 properties both of the prosthesis itself and of the duct to be regenerated.

The activation of the prosthesis was performed with an Ar cold plasma, by operating at 50 W, 3 minutes, at the temperature of 20°C. The activated prosthesis was then sterilised and seeded with endothelial cells, with the methodology described in the example 9, before
being implanted.

15 The invention as it is exemplified above is susceptible to numerous modifications and variations all falling within the ambit of protection of the following claims.

CLAIMS

1. A process of surface activation of products for biomedical use based on biocompatible and bioabsorbable aliphatic polyesters selected from a group formed by homo and copolymers derived from L-lactide, D-lactide, D,L -lactide, racemic D,L-lactide, meso D,L-lactide, ϵ -caprolactone, glycolide, δ -valerolactone, preferably copolymers of L-lactide, D-lactide, racemic D,L-lactide, meso D,L-lactide with ϵ -caprolactone, which consists in carrying out at least one of the following treatments: acid or base hydrolysis, treatment by cold plasma, or by chemical reactions or with electromagnetic radiations.
2. A process as claimed in claim 1, characterised in that the surface activation by means of hydrolysis can be carried out at temperatures preferably comprised between 0°C and the softening temperature of the material.
3. A process as claimed in claim 2, characterised in that said aliphatic polyesters comprise 50/50 poly(L-lactide-co- ϵ -caprolactone) and the hydrolysis is conducted at temperatures comprised between 5°C and 80°C, preferably between 30°C and 50°C.
4. A process as claimed in anyone of the claims from 1 to 3, characterised in that said hydrolysis treatment is conducted in the presence of an aqueous solution of a base.
5. A process as claimed in claim 4 characterised in that said base is a strong alkali.
6. A process as claimed in claim 5, characterised in that said strong alkali is selected from a group formed by NaOH, KOH, Na₂CO₃, LiOH, Ca(OH)₂.
7. A process as claimed in claim 4, characterised in that said base is a weak base.
8. A process as claimed in claim 7, characterised in that said weak base is NH₄OH.
9. A process as claimed in claim 7 or 8, characterised in that in that said weak base comprises at least one amine.
10. A process as claimed in claim 9, characterised in that said amine comprises at least one amine selected from a group formed by methylamine, ethylamine, diethylamine and dimethylamine.
11. A process as claimed in anyone of claims from 1 to 3, characterised in that said hydrolysis treatment is conducted in the presence of an aqueous solution of an acid.
12. A process as claimed in claim 11, characterised in that said acid is selected from a group formed by HCl, HClO₃, HClO₄, H₂SO₃, H₂SO₄, H₃PO₃, H₃PO₄, HI, HIO₃, HBr, lactic acid, glycolic acid.
13. A process as claimed in any one of claims from 2 to 12, characterised in that the

surface hydrolysis is followed by washing for removing any trace of acid or base.

14. A process as claimed in claim 1, characterised in that said plasma treatment is carried out in a presence of a reactive gas for a formation of surface activations of oxygenate type.
- 5 15. A process as claimed in claim 10, characterised in that said reactive gas and air and said surface activations of oxygenate type are -OH, -CHO, -COOH and the like.
16. A process as claimed in claim 14, characterised in that said plasma treatment is carried out in a presence of argon.
17. A process as claimed in claim 14, characterised in that said plasma treatment is carried
10 out with oxygen.
18. A process as claimed in claim 17, characterised in that said plasma treatment is carried out with air.
19. A process as claimed in any one of claims from 2 to 18, characterised in that the hydrolysis or the plasma treatment is followed by a phase of induction of further
15 chemical modifications.
20. A process as claimed in claim 19, characterised in that -COONa groups generated by base hydrolysis are converted into -COOH groups by means of treatment with strong mineral acids.
21. A process as claimed in claim 19, characterised in that a surface freeing of alcoholic
20 and/or carboxylic and/or aldehydic groups by means of a hydrolysis process is followed by a chemical reaction by means of addition of a compound provided with an activated group or groups able to react with respective surface alcoholic and/or carboxylic and/or aldehydic groups.
22. A process as claimed in claim 21, characterised in that said compound comprises
25 succinic anhydride, which causes a conversion of -OH groups into -O-CO-CH₂-CH₂-COOH groups.
23. A product for biomedical use based on surface-activatable biocompatible and bioabsorbable aliphatic polyester selected from a group formed by homo and copolymers derived from L-lactide, D-lactide, racemic D,L-lactide, meso D,L-lactide, ϵ -caprolactone, glycolide, δ -valerolactone, preferably copolymers of L-lactide, D-lactide, racemic D,L-lactide, meso D,L-lactide with ϵ -caprolactone, for obtaining
30 prostheses.
24. A product for biomedical use as claimed in claim 23, characterised in that said

aliphatic polyester comprises homo and copolymers of lactic acid.

25. A product for biomedical use as claimed in claim 24, characterised in that said aliphatic polyester comprises copolymers of L-lactide, D-lactide, D,L-lactide with ϵ -caprolactone surface-activated with COONa, -COOH, OH groups.
- 5 26. A partially bioabsorbable vascular prosthesis, comprising a product as claimed in anyone of claims 23 to 25, the surface of which is activated by means of a process according to anyone of claims 1 to 22.
27. A prosthesis as claimed in claim 26, characterised in that it comprises a reinforcement sheath, advantageously made of Dacron®.

1 / 4



Fig 1a

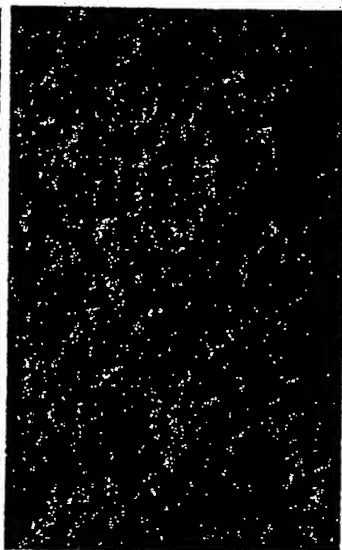


Fig 1b



Fig 1c



Fig 2

2 / 4 .

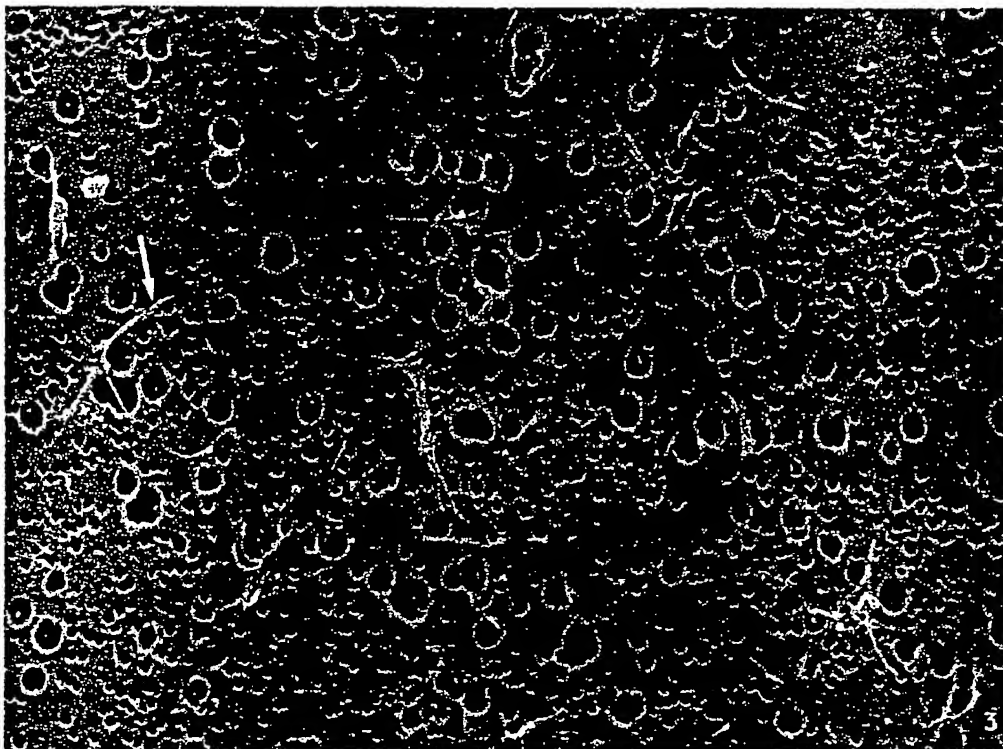


Fig 3

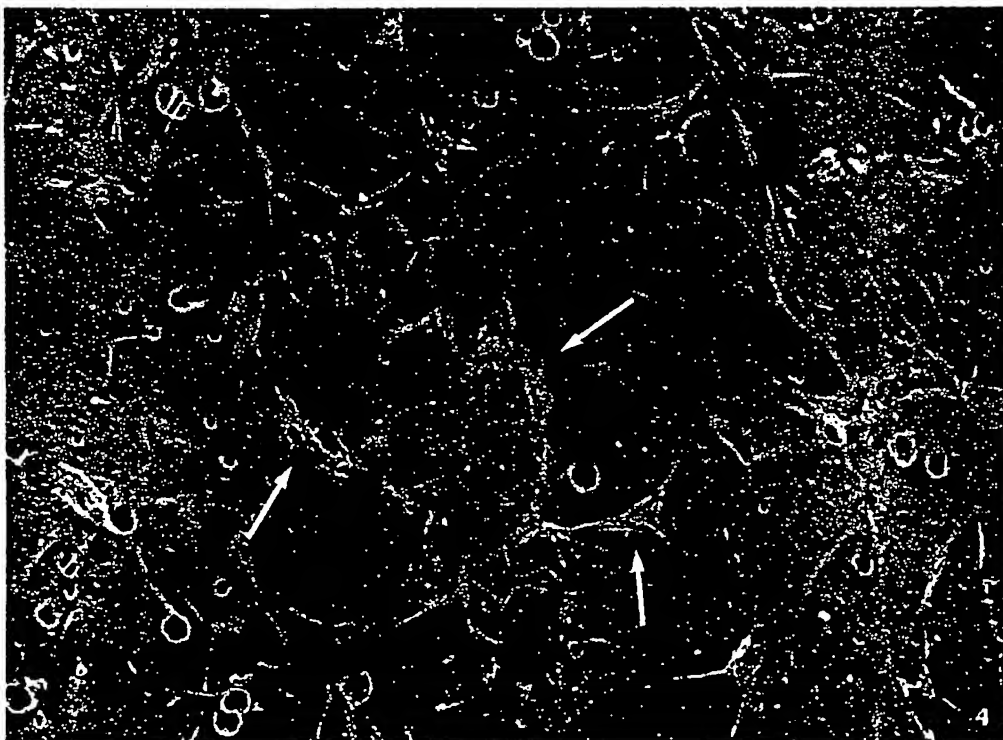


Fig 4

3 / 4

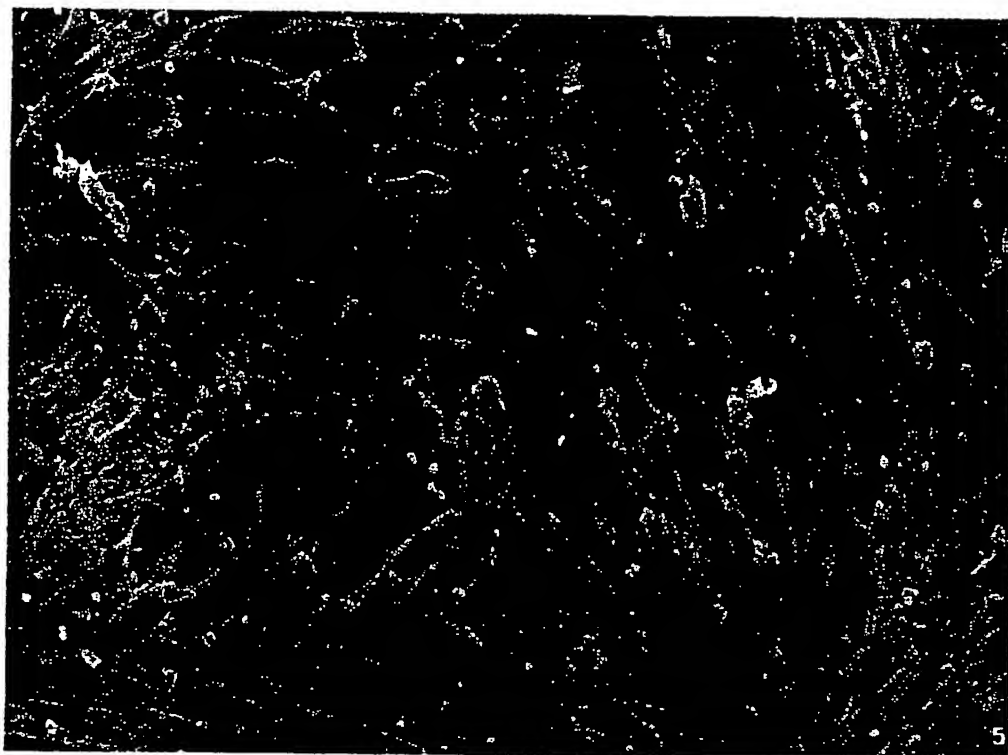


Fig 5

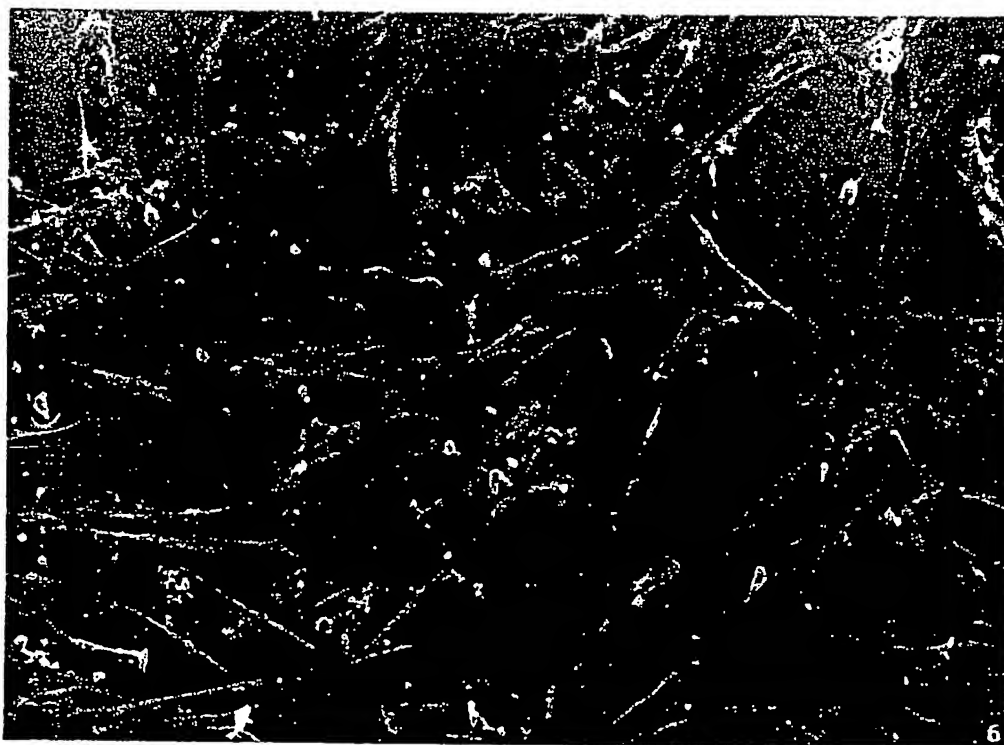


Fig 6

4 / 4

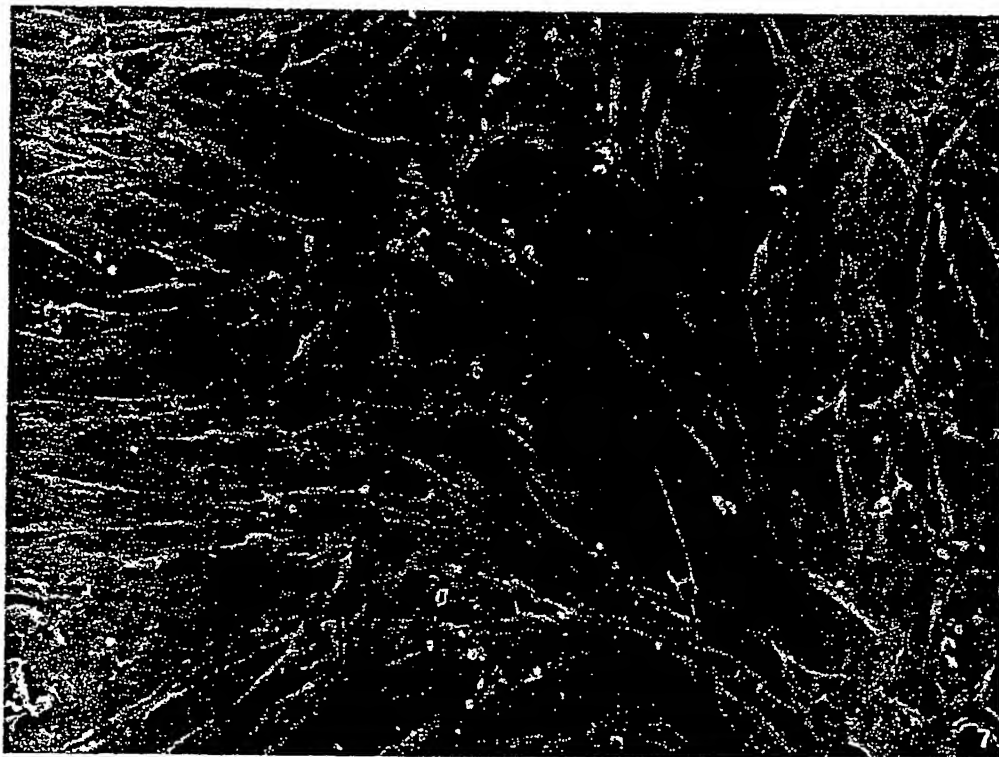


Fig 7

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IT 96/00149

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C08J7/12 A61L27/00 //C08L67/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C08J A61L C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB,A,2 141 435 (AMERICAN CYANAMID CO) 19 December 1984 see claims 1,8,9,11 see page 6, line 42 - line 72 ---	1,2,23
X	EP,A,0 358 819 (DOW CORNING KK) 21 March 1990 see claims 1,3,5,8 see page 3, column 4, line 12 - line 17 see page 3, column 4, line 58 - page 4, column 5, line 15 ---	1,2
A	US,A,5 236 563 (LOH INH-HOUNG) 17 August 1993 see claims 1,11-13 --- -/--	1,23,24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

19 December 1996

Date of mailing of the international search report

06.01.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+ 31-70) 340-3016

Authorized officer

Niaounakis, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IT 96/00149

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 558 965 (AMERICAN CYANAMID CO) 8 September 1993 see claims 1-3,6,10 see page 9, line 16 - line 39 ---	1
A	GB,A,2 167 665 (UNIV LIVERPOOL) 4 June 1986 see claims 1,5,6,12,17 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IT 96/00149

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB-A-2141435	19-12-84	US-A- 4496446	29-01-85
		AU-B- 569891	25-02-88
		AU-A- 2943384	20-12-84
		BE-A- 899939	18-12-84
		CA-A- 1247041	20-12-88
		CH-A- 667586	31-10-88
		DE-A- 3422160	20-12-84
		FR-A- 2548904	18-01-85
		HK-A- 10992	31-01-92
		JP-B- 6024590	06-04-94
		JP-A- 60012068	22-01-85
		NL-A,B,C 8401926	16-01-85
		SE-B- 461794	26-03-90
		SE-A- 8403239	17-12-84
EP-A-0358819	21-03-90	CA-A- 1322262	21-09-93
		JP-A- 64002643	06-01-89
		AU-B- 609867	09-05-91
		AU-A- 2189588	15-03-90
		US-A- 4882162	21-11-89
US-A-5236563	17-08-93	NONE	
EP-A-0558965	08-09-93	US-A- 5352515	04-10-94
		AU-B- 671976	19-09-96
		AU-A- 3391793	09-09-93
		CA-A- 2090565	03-09-93
		JP-A- 6041310	15-02-94
		PL-A- 297901	15-11-93
		US-A- 5442016	15-08-95
		US-A- 5530074	25-06-96
GB-A-2167665	04-06-86	ZA-A- 9301445	23-09-93
		DE-A- 3542703	05-06-86

Figure 1 is a microphotograph obtained by scanning electron microscope (SEM) at a magnification of 1500x, which illustrates the surface morphology of a disc in non-activated poly(L-lactide-co-ε-caprolactone) prepared as above-specified.

Example 3

Activation by means of base hydrolysis at 40°C

80ml of dilute solution of NaOH at 5% were placed in a 100ml flask, and a disc of 50/50 poly (L-lactide-co-ε-caprolactone) having a thickness of 500 micron and a diameter of 22mm was prepared, as described in example 1. The flask was immersed in a thermostated water bath at 40°C, and stirred by an electromagnet.

These conditions were maintained for 18 hours, then the alkaline solution was removed and the whole was repeatedly washed with distilled water for four hours. The discs of the material thus activated exhibited a wetting angle with water of 30°, measured at 23°C in air. The inherent viscosity of the material, determined in chloroform at 25°C, at a concentration of 0.2g/dl was 3.00dl/g.

Example 4

Activation by means of base hydrolysis at 20°C

80 ml of dilute NaOH at 5% were placed in a 100ml flask and a 50/50 poly(L-lactide-co-ε-caprolactone) disc having a thickness of 500 micron and a diameter of 22mm was prepared, as described in example 1. The flask was kept at a temperature of 20°C, slowly stirring the contents by means of an electromagnet. These conditions were maintained for 18 hours, then the alkaline solution was removed and numerous washes with distilled water were carried out over a period of 4 hours.

The discs of material thus activated exhibited a wetting angle with water of 48°, measured at 23°C in air. The inherent viscosity of the material, determined in chloroform at 25°C at a concentration of 0.2g/dl was 3.02dl/g.

Figure 1b is a microphotograph obtained by scanning electron microscope (SEM) at a magnification of 1500x, which illustrates the surface morphology of a disc in poly(L-lactide-co-ε-caprolactone) activated by base hydrolysis with NaOH according to the present example.

Example 5

Conversion of -COONa surface groups into -COOH groups.

80 ml of 3% HCl solution were placed in a 100ml flask and a 50/50 poly(L-lactide-co-ε-caprolactone) disc having a thickness of 500 micron and a diameter of 22mm was